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IRRADIATION INJURY OF BONE TISSUE

A vital microscopic method

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Numerous reports (for review see NATHANSSON 1977) deal with the effect of irradiation on bone tissue, but many central questions remain unsolved, as for instance whether bone necrosis after irradiation is preceded by a vascular injury. Histology and microangiography after irradiation are indirect methods and provide no repeated evaluation of the events occurring in the injured bone. Only a direct method also enabling a repeated observation of the same tissue compartment at different stages after a defined irradiation dosage, would allow more definite conclusions concerning the effect of irradiation. The titanium bone chamber is an attempt to overcome drawbacks connected with the indirect methods. Using the bone chamber, vital microscopy of bone tissue and vessels becomes possible both before and at repeated intervals after irradiation. Direct comparison can thus be performed and the development of the tissue injury can be recorded in situ. Previous vital microscopic methods, applied to the examination of soft tissues, such as the rabbit ear chamber (SANDISON 1924) or the mouse skin chamber (ALGIRE 1943), have also been used on grafted bone tissue (SANDISON 1928, KIRBY-SMITH 1933, CLARK & CLARK 1942, KIEHN et coll. 1952, EZRA-COHN et coll. 1969, SUDMANN 1975). These methods, although allowing a repeated observation of bone tissue changes in the living animal, are limited to heterotopical bone grafts. No evaluation of the behaviour of bone tissue in situ is possible.

However, this can be performed using the titanium bone chamber now described.

Material and Methods

Bone chamber and recording procedures. The titanium bone chamber (Fig. 1 a) consists of a hollow screw into which one short and one long glass rod are glued leaving a 100 micron wide space in-between. If the chamber is inserted into a long bone of a rabbit, using a gentle surgical technique, bone and vascular tissue will grow into this space during a healing period of 4 to 6 weeks. With the animal in light anaesthesia it is then possible to observe the ingrown tissue in the vital microscope. The bone and vascular structure are recorded on film and slides.

Vital microscope. A modified Leitz intravital microscope was used for all in vivo recordings. As a rule, magnifications between 20× and 100× were tried. A Vinten scientific 16 mm film camera and a Leica camera were connected to the vital microscope. Kodachrome 25 cine film and Ektachrome 200 film were used.

Animals and anaesthesia. Preliminary experiments were performed on 5 healthy adult rabbits (Belgian hare), 10 to 16 months of age, weighing 4.5 to 6.0 kg. During surgery, irradiation, and vital

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microscopic sessions, the animals were under general anaesthesia maintained by intramuscular injections of Hypnorm (Mekos, Helsingborg, Sweden) at a dose of 0.7 ml/kg body weight. The Hypnorm drug is a combination of Fentanyl and Fluanison.

Irradiation procedure. The rabbits were irradiated at a temperature of 22°C. During the irradiation the bone of the animal was placed upon a 10 cm thick polystyrene phantom. A ^{60}Co unit (Siemens Gamma-tron G3) was chosen to minimize the difference in the absorbed dose in soft tissue and bone and in the disturbances in the radiation field due to the titanium chamber. Source skin distance was 60 cm and the field size was 5 cm \times 5 cm. The dose rate was 1.20 Gy/min. A 5 mm bolus of silicone rubber material was applied to ensure full build-up as the top of the bone chamber was only 2 mm below the skin surface (Fig. 2). Each rabbit was given 15.0 Gy (the absorbed dose in water at 5 mm, corresponding to the position of the chamber). The appearance of the bone was recorded during the healing period following surgery, and 2 weeks later. The irradiation was then performed (Fig. 1b). Further recording was performed each fourteenth day until the animal was killed.

Histologic technique. After killing of the animal, fixation of the bone in formaldehyde, and decalcification in formic acid, the tissue in the chamber space was removed, embedded in paraffin, cut transversally and stained with HTX-eosin.

Results and Discussion

The woven bone before irradiation (Fig. 3 a) became subsequently lamellated (Fig. 3b). This process involves reorganization of the collagen fibres and the osteocytes of the bone (ALBREKTSSON 1980). In principle, woven bone maturation does not seem to be influenced by the irradiation, at least not during the first weeks. At about 4 weeks after the irradiation (Fig. 3 c) the mature bone tissue was slightly remodelled as demonstrated by the changed appearance of the islands of connective tissue proper in the bone. This bone remodelling occurred at a similar rate and to the same extent as in normal bone in a series of 70 non-irradiated rabbits (ALBREKTSSON 1979). At 6 weeks after injury (Fig. 3 d) the vascularity changed and a number of wide venular vessels appeared in the bone tissue. Bone

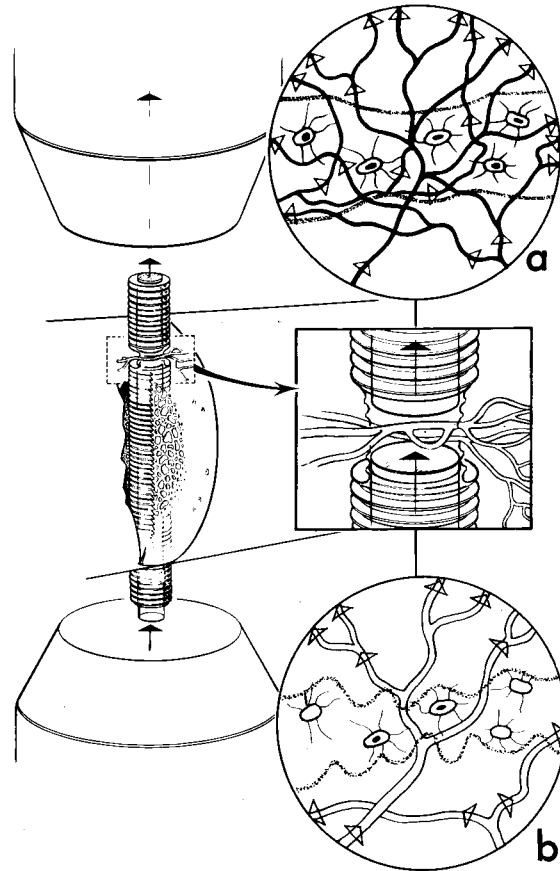


Fig. 1. a) Tissue grown into the chamber space (enlarged section) before irradiation. The rounded drawing is a schematic representation of the chamber microscopic image. Each osteocyte is thought to represent a nutritive bone unit. b) Repeat intravital recording at 2 weeks after irradiation.

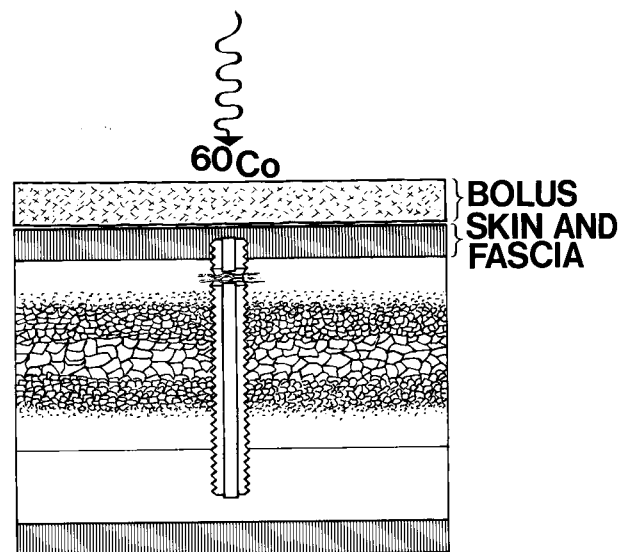
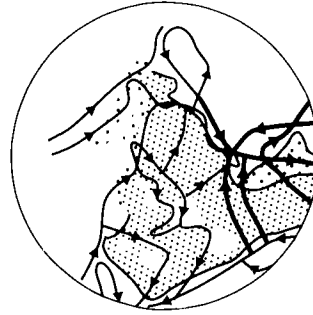
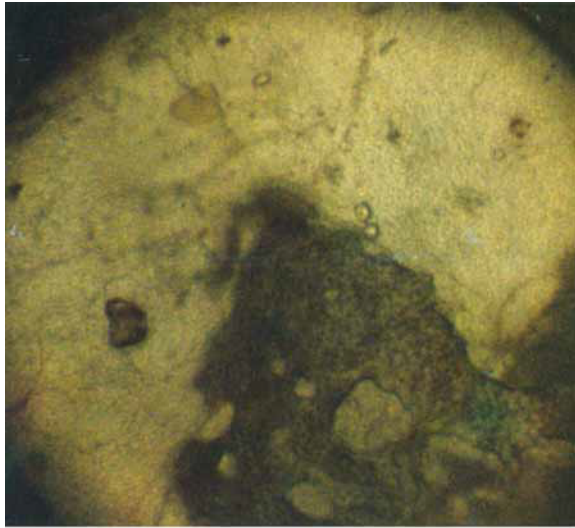
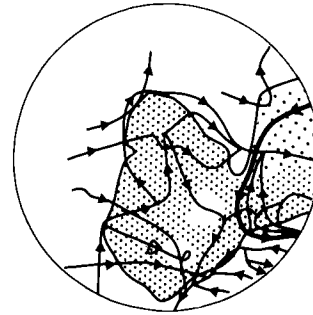
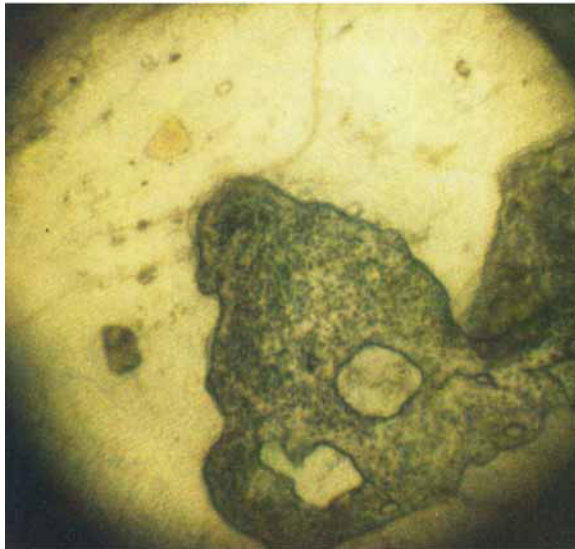


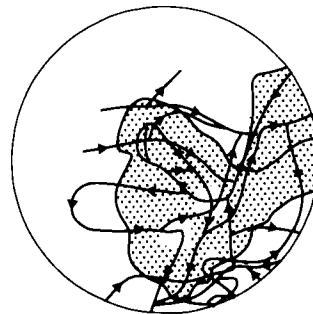
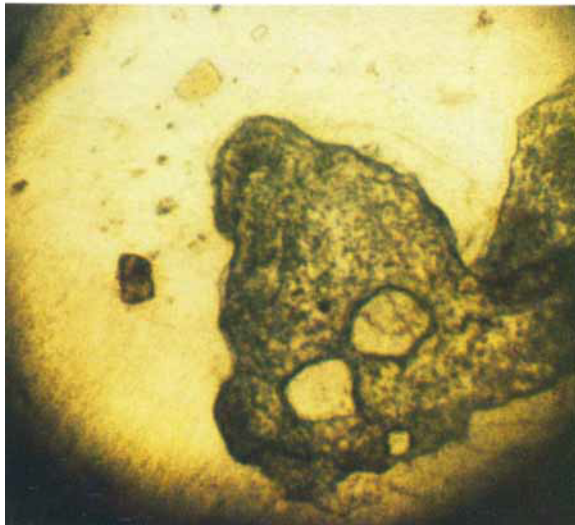
Fig. 2. A 5 mm bolus of silicone rubber was applied at the irradiation.



a) Conditions before irradiation. Osteogenesis is indicated by woven bone in some places.



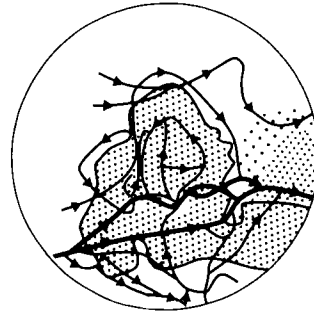
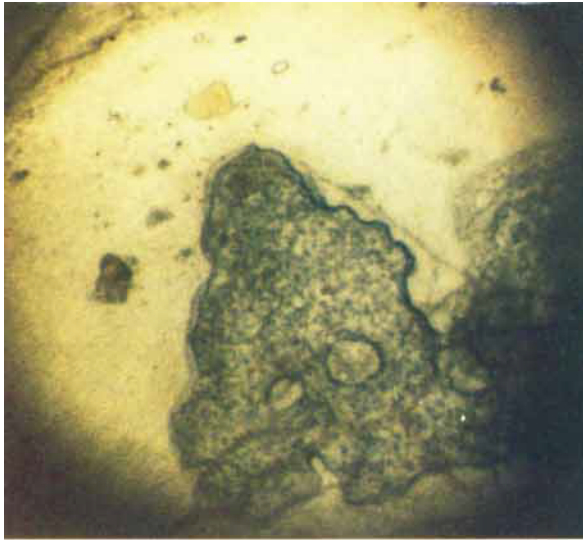
b) 2 weeks after irradiation. The former woven bone in the left part of the bone tongue is now mature as indicated by a clear outer demarcation line and normal osteocytes.



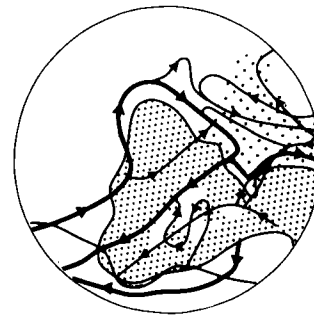
c) 4 weeks after irradiation all bone in the chamber is considered mature. Up till now the remodelling of the bone is quite normal.

Fig. 3. In the explanatory sketch close dots represent mature bone and spaced dots woven or immature bone. Arrows on

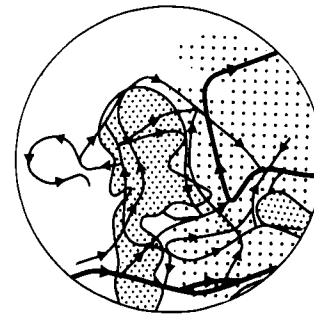
vessels indicate direction of blood flow. 15 Gy were given at irradiation.



d) 6-week wide venule, immature bone appears again and more active bone resorption starts.



e) 8 weeks after irradiation bone resorption is widespread and immature bone is further developing.



f) 10 weeks after irradiation most of the original bone is resorbed. The chamber image is instead dominated by immature bone still with no outer demarcation or a clear representation of osteocyte lacunae.

Fig. 3. (For legend see preceding page.)

resorption began. At 8 weeks (Fig. 3 e) resorption of the original bone was more apparent, and at 10 weeks most of the original bone was resorbed. The findings of wide venules appearing before beginning of bone resorption should not be considered as implying that the injury to the vessels occurred more rapidly than that to the bone. It has previously been observed (ALBREKTSSON 1980) that bone resorption normally is associated with wide vessels and slow blood flow. This means that the wider venules observed might just as well reflect a vascular response to abnormal bone, as a primary vascular injury due to irradiation. Simultaneously with the onset of bone resorption, the development of new bone tissue was obvious (Fig. 3 d, right part of the observation field). This new bone tissue rapidly spread over the chamber field during the following weeks (Fig. 3 e-f). The vital microscopic view of this bone resembled that of woven bone which, however, normally would become lamellated during a time lapse of 6 weeks. This new bone still lacked definite outer demarcation and clearly established osteocyte lacunae at 10 weeks.

The present experiments have shown that the titanium chamber is a well functioning method for observing and recording bone injury following irradiation. The vital microscopic approach allows a demonstration of the different stages in the development of an irradiation injury. The method has been applied in further experiments on the effects of different types of irradiation injury to bone tissue.

SUMMARY

A vital microscopic method using the titanium chamber for observation of bone irradiation injury in situ is described. The subsequent development of bone tissue

resorption and replacement with a pathologic immature bone could be observed and recorded.

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